Remarks/Arguments

Claims 1-13 are pending in the application. Claims 1-7 and 12-13 have been withdrawn from consideration pursuant to a lack of unity objection. Claims 8-11 are under consideration.

Election / Restriction Requirement

Reconsideration and withdrawal of the lack of unity objection as to all claim groups is respectfully requested. As pointed out in the Response to Restriction Requirement, the special technical feature of the invention is an immunogenic determinant which comprises one or more complexes of a heat shock protein or stress protein bound with an antigenic peptide fragment the foregoing being extracted from a cell infected with an intracellular bacterial, protozoan or parasitic parasite, with this complex being derived from a stressed cell. That feature is neither disclosed or rendered obvious by Srivastava.

Srivastava discloses that stress protein/peptide complexes which are isolated from cells are immunogenic. However, these complexes are constitutively expressed and do not result from the stressing of the cell (for example by heat). This is an important distinction as the stress protein/peptide complexes of the present invention, when used as the immunogenic determinant in a vaccine are shown to be more immunogenic and also shown to induce improved long term immunity. The improved immunity which is obtained from using stress protein/peptide complexes which are derived from stressed cells over that of stress protein/peptide complexes from constitutively expressed cells is exemplified in Examples 4 and 5 of the present Application.

Example 4 uses CZE (capillary zone electrophoresis) profiling to illustrate that peptides bound by heat stressed, TNF (cytokine) stressed and constitutive (non-stressed) stress proteins are significantly different from each other (as shown in Figures 1-3). Further, when the heat induced, TNF induced and constitutive stress protein/peptide complexes were used to immunize an animal, the heat induced stress protein/peptide complexes were shown to produce the highest antibody titre.

Further, Example 5 provides a comparison of constitutive and TNF-induced stress protein/peptide complexes on inducing immunity in stress proteins. Animals vaccinated with TNF-induced stress proteins showed a 10 to 100 fold higher antibody titre than those immunized with constitutive stress proteins.

Thus, it is apparent that the induced stress protein / peptide complexes of the present invention are not the same as the constitutively expressed stress protein / peptide complexes of Srivastava. The former qualifies as a special technical feature as defined b PCT Rule 13.2. The feature is common to all claims. Thus, withdrawal of the lack of unity objection is respectfully requested, along with rejoinder of Claims 1-7 and 12-13 for examination with Claims 8-11.

Drawings

Examiner alleges that the drawings are informal. Applicants did not declare the drawings informal. The application represents the US national phase of a PCT application. The drawings of the PCT application are formal. Hence, the drawings of the national phase are by definition also formal.

It is further noted that no notice of review by the Official Draft Persons has been provided, pointing out any deficiency in the drawings.

Reconsideration and withdrawal of the requirement for further "formal" drawings is respectfully requested.

Specification

In response to section 7 and 8 of the Detailed Action, the specification has been amended to change the spelling of "immunised" and "immunisation" to *immunized* and *immunization*, respectively. The term 'Quil A' has been capitalized with "TM" placed in superscript thereafter to denote its use as a Trade Mark. The description "Quil A Saponin" has been included in

parentheses. The first occurrence of the term 'RPMI' has been explained by the inserted parenthetical expression "Roslin Park Medical Institute".

Priority

Examiner's request for amendment of the specification to include a reference to an earlier application is incorrect. The prior applications which must be referenced are prior US nonprovisional applications from which internal priority is claimed pursuant to 35 USC 120 (continuations, continuations-in-part, and divisionals), or US provisional applications, from which priority is claimed pursuant to 35 USC 119(e). The instant application comprises the US national phase of a PCT application. It is not a continuation, continuation-in-part, or divisional of an earlier US application; nor does it claim the benefit of the filing date of an earlier US provisional application. Thus, amendment of the specification is not required under Rule 78.

Double Patenting

Claims 8-11 have been provisionally rejected for obviousness-type double patenting over claims 11-15 of copending Application No 10/049,704 ('704 Application).

Applicant submits that there is a distinct and appreciable difference between the subject matter of the instant Application and those of the '704 Application, and that accordingly the claims are patentably distinct.

In the instant Application, the immunogenic determinant is comprised of a stress protein complexed with a peptide, where the stress protein and peptide (antigen) are derived from a stressed cell. In the present case, the cell is a eukaryotic cell. This cell would be infected by an *intracellular* pathogen in accordance with the claims of the instant invention. Upon stressing the cell, the production of stress proteins would be up regulated within the cell. These stress proteins would form complexes with peptides derived from the intracellular pathogen, this forming a

stress protein/peptide complex in which the stress protein is derived from the eukaryotic cell and the peptide is derived from the intracellular pathogen.

The claims which are subject of the '704 Application relate to a stress protein/peptide complex formed from an extra-cellular pathogen. It is inherent in the nature of the extra-cellular pathogen that it does not infect a cell. The immunogenic determinant of the '704 Application comprises a complex of a stress protein which is derived from the extra-cellular pathogen which is in a complex with a peptide which is also derived from the extra-cellular pathogen. Accordingly, both the stress protein and the complexed peptide of the '704 Application are derived from the extra-cellular pathogen.

As outlined above, it is therefore submitted that immunogenic determinants as claimed in the instant Application and the '704 Application are structurally different. The allegedly conflicting claim are patentably distinct. Reconsideration and withdrawal of the provisional obviousness-type double patenting rejection is respectfully requested.

Response to Section 112, 1st paragraph Rejection

Examiner alleges that while the specification is deemed enabling for an immunogenic composition comprising a complex of a shock protein and an antigenic peptide, it is not enabling for a vaccine.

With regard to the points on enablement, it is pointed out that assessment of enablement is not based on whether any experimentation is necessary to perform the scope of the invention as defined in the claims, but rather whether the level of that experimentation is undue. Importantly, the fact that experimentation may be complex does not make it undue.

In the present case, the person of ordinary skill in the art would be substantially qualified and experienced in the field, having at least a post-doctoral level of qualification.

A vaccine composition in accordance with the instant invention is described at page 6, lines 1 to 8 as containing an immunogenic determinant (the stress induced heat shock protein-peptide complexes) and, typically, an adjuvant. Page 10, line 31 to page 11, line 7 provides specific guidance on the specific composition of a suitable vaccine preparation. Specifically, the vaccine will contain the immunogenic determinant, and typically an adjuvant and an aqueous carrier.

Suitable adjuvants are listed on page 6 at lines 13 to 15 and also at page 11, line 6. The concentration of the heat shock protein /peptide complex within the vaccine composition is discussed from page 11, line 30 to page 12, line 2. Specific dose levels in relation to body weight are provided.

The immunogenic determinant is produced by stressing a cell infected with an intra-cellular pathogen. Any suitable pathogen-infected cell can be used in the present invention to provide a source of heat shock protein / peptide complexes. Stressing the cell in order to induce heat shock protein production is achieved by stressing the cell with either heat or tumor necrosis factor. It is particularly preferred that where a cell is infected with a protozoan or parasitic pathogen that the stress induction is by tumor necrosis factor (see description – page 7, lines 9 to 12). Where the cell is infected with a bacterial pathogen, it is preferred that stress induction is by heat treatment of infected cells (page 7, lines 12 to 16). It should be noted however that page 8, lines 14 to 17 teaches the reader that the exact form of stress treatment is not crucial to the invention, rather the treatment need only result in the stimulation of the production of the desired immunogenic complexes within the stressed cells.

The stressing of the cell is an essential step as this results in the formation of stress protein/peptide complexes which are more immunogenic that constitutive stress protein/peptide complexes. Such "induced" stress protein/peptide complexes bind a different profile of peptides than those bound by constitutive stress protein/peptide complexes. The "induced" stress protein/peptide complexes also produce improved immunity following immunization (see Examples 4 and 5 of the instant Application).

In order to produced the vaccine of the present invention, the stress protein/peptide complexes (immunogenic determinants) must be extracted and purified from the stressed cells. Although a variety of extraction techniques may be used, with these being well-known to the skilled artisan, a specific example of one extraction method for stress protein/peptide complexes is provided at page 10, lines 5 to 18.

Administration of the resulting vaccine may be by a number of routes, with these being standard to those skilled in the art and listed for convenience on page 11, lines 20 to 26.

The subsequent Examples provided in the specification further exemplify the guidance relating to vaccine production and administration which is discussed above.

In Example 1, induced heat shock protein(stress protein)/peptide complexes from *M. Bovis* are exemplified. Heat shock is used as a stress stimulus to induce heat shock protein/peptide formation. Ample guidance on extracting the heat shock protein/peptide complexes from the stressed cells is provided. Additional methodology relating to the purification of the heat shock protein/peptide composition from the stressed cell is provided in the last paragraph of page 13.

Example 2 demonstrates the induction of stress protein/peptide complexes in a cell infected with Plasmodium. In order to induce stress protein production, the cell is incubated with tumor necrosis factor, more particularly tumor necrosis factor alpha. The resulting heat shock protein/peptide complexes were extracted from the cell using the same methodology as described in Example 1.

Example 3, relates to immunization of mice and rabbits with a vaccine composition prepared in accordance with either Example 1 of the invention (a bacterial intra-cellular pathogen which has been stressed using heat) or by Example 2 (where a cell line infected with plasmodium is stressed with tumor necrosis factor). In each of the two vaccines produced, the level of the heat

shock protein/peptide complex exemplified as being provided into the vaccine is within the preferred ranges set forth on page 11, lines 30 to 31.

A booster dose of each vaccine was administered one month after the initial immunization injection. This booster comprised a vaccine of identical composition to the vaccine as initially administered. In order to assess whether the vaccine had induced immunity, and in particular long term protective immunity desired, the vaccinated animals were challenged with the pathogen which was infecting the cell line from which the heat shock protein/peptide complexes were originally derived. This challenge was made at a period of 6, 12 and 18 months following the initial immunizations. The measured antibody responses illustrated that protective immunity had resulted from each vaccination course.

Example 4 shows that stress protein/peptide complexes which result from a cell being specifically stressed result in stress protein/peptide complexes being produced which are induced as opposed to being constitutively expressed as part of the normal peptide sampling and display functions of the cell. These induced stress protein/peptide complexes bind a different profile of peptides to constitutively produced stress proteins and confer increased immunity when incorporated into a vaccine. Immunization with a vaccine containing induced stress protein/peptide complexes confers a higher level of immunity against the pathogen from which the stress protein/peptide complex is derived from over that conferred by a non-induced stress protein/peptide complexes (i.e. those constitutively expressed in the cell as part of normal cellular function).

The vaccines of the present invention may be applicable to any intracellular pathogen (p. 7, lines 11 to 14). What constitutes an intracellular pathogen would be well known to the person skilled in the art.

Stress proteins (heat shock proteins) are one of the most conserved families of proteins found in nature. As referenced at page 1, line 27 and page 6, lines 20 to 30.

The methods of the present invention can be applied to any intracellular pathogenic organisms as defined in the description (namely bacteria, prokaryotes and protozoa). The high level of conservation known to exist in heat shock protein structure, expression and function across different species, and in particular throughout the intracellular pathogens listed in relation to the instant application means that the skilled man could apply the techniques of the present invention to formulate a vaccine composition for use against any intracellular pathogen. In preparing such a vaccine, the skilled man would readily expect to obtain the described immunogenic determinant, specifically the induced stress protein/peptide complex which could be incorporated into a vaccine in accordance with the teachings of the specification. The skilled man would be expected to be able to readily administer the vaccine to an animal, preferably a human, in order to illicit immunity against the intra-cellular pathogen which infected the cell from which the heat shock protein/peptide complex used in the vaccine was initially derived.

Stressing a cell infected with any intra-cellular pathogen in accordance with the methods provided in the invention would result in an induced stress protein/peptide complex. The complex can be isolated and incorporated into vaccine composition as the immunogenic determinant and used to induce protective immunity. The instant description provides clear guidance to the skilled man as to how a cell infected with an intracellular pathogen can be stressed. The description also provides clear guidance on isolating / purifying the stress protein/peptide complex for use as a vaccine component.

With reference to the Examiner's specific comments at the top of page 6 of the Official Action, the compounds of the present invention include "antigenic proteins" in accordance with the cited definition of the term "vaccine" provided by the Examiner from Dorland's Medical Dictionary.

It should be noted however that the principals which underlie the use of a vaccine which contains stress protein/peptide complexes as the immunogenic determinant necessarily require that the antigenic peptide of the pathogen which is used in the vaccine to induce immunity will not be

limited to a single, unique peptide. Rather, due to the nature of heat shock proteins in binding a variety of peptides, the stress protein/peptide complexes of the instant invention will provide a number of antigenic peptides from the pathogen for use in inducing immunity.

The presence and conservation of the heat shock family of proteins throughout species and in particular throughout all intracellular pathogens means that the method and composition of the instant invention can be applied to all bacterial, protozoan and parasitic cases as the use of the instant invention to induce immunity against any or all of these pathogens or their resulting infectious diseases would comprise the same standard steps of

(i) inducing stress protein/peptide complex formation by stressing cells infected with intra-cellular pathogens using any of the methods described in the specification;

(ii) extracting the immunogenic determinant in accordance with the guidance provided, for example, in Example 1;

(iii) formulating a vaccine composition containing at least the immunogenic determinant, and adjuvant selected from the list provided and aqueous solution, with the immunogenic determinant being added at an amount set forth in the specification at page 11, line 30 to page 12, line 2; and

(iv) administering the vaccine composition to an individual in need of protection against the intracellular pathogen.

It is therefore specifically submitted that the applicant's invention is enabled for the prevention, amelioration or treatment of all bacterial, protozoan or parasitic diseases as claimed, due to conserved nature and function of stress proteins and further due to the uniform steps and standard methodology required to produce a vaccine.

It is further submitted that the description does provide the requisite level of enablement in order to support the claims presently on file. With regard to Examiner's remark that in connection with vaccine and dosage frequency, it is submitted that there is sufficient guidance provided in the

description relating to the vaccine, its composition and dosages. Examples 1 and 2 of the present invention teach of the preparation of the vaccine and in particular the immunogenic component.

Further, Example 3 describes the dosing regimen for the vaccine. This is specifically stated as being administered at a level of from 1 to 10 micrograms of the immunogenic complex in a phosphate buffered saline solution. Subsequent to this initial administration, a booster (with an identical vaccine preparation and dosage) was provided at a period of one month after the initial inoculum.

The vaccines which may be used in Example 3 are prepared as described in Example 1 (preparation of immunogenic component using heat shock) and Example 2 (preparation of immunogenic component using cytokine). In order to assess whether the vaccine induced protective immunity, rabbits were subsequently challenged with the target pathogen (i.e. the intracellular pathogen which initially infected the stressed cell). This challenge was made at 6, 12 and 18 months after the initial immunization and served to illustrate that a memory response had been generated.

Accordingly it can be seen that the vaccine produced according to the methods of Examples 1 or 2 and administered as per the using the dosage and administration method of Example 3 provides an immune response and long term immunological memory.

Turning to the Examiner's comments at the foot of page 6 with regard to the correlation of the vaccine of the present invention with equivalent results in humans, Applicant respectfully submit that the 1989 scientific journal paper cited by the Examiner in support of the rejection does not actually reflect a state of art at the priority filing date of the present application namely, August 1999.

Submitted herewith a copy of the 1998 paper, Baldwin et al., "Evaluation of New Vaccines in the Mouse and Guinea Pig Model of Tuberculosis", *Infection and Immunity, 66(6)*:2951-2959,

which exemplifies vaccine models in the mouse and guinea pig in relation to immunity from tuberculosis.

Baldwin et al. demonstrates that vaccination in the animal model can be used as a basis from which to extrapolate equivalent expected results in the human model. The paper serves to confirm that the skilled artisan in the field would understand this as being so.

It is further pointed out that an application to the NIAD which included only data for a tuberculosis vaccine conducted on animals would be considered by the NIH to provide sufficient information in order to consider the efficacy of that vaccine. The journal paper of Brennan et al., "Propelling novel vaccines directed against tuberculosis through the regulatory process", *Tubercle and Lung Disease (1999) 79(3)* 145-151 provides specific guidance on this point. The authors are affiliated with the USFDA's Laboratory of Mycobacteria. The article provides guidance on the procedure for the development and testing of new tuberculosis vaccines and specifically how new tuberculosis vaccines can be progressed through clinical trials. Importantly, the authors speak to the usefulness and predicative value of animal models, and the mouse aerosol model in particular. See page 148, column 2 and page 149.

To further support the fact that the stress protein/peptide complexes of the present invention can be used to induce immunity against tuberculosis, a recently published paper by the inventor is provided. This paper, "BCG (Bacille Calmette-Guerin) HspCs (heat-shock protein-peptide complexes) induce T-helper 1 responses and protect against live challenge in a murine aerosol challenge model of pulmonary tuberculosis" *Biochemical Society Transactions (2004) Vol 32, part 4*, shows that stress proteins/peptide complexes are effective as tuberculosis vaccines, as they successfully induced immunity.

Response to Section 112, 2nd paragraph Rejection

Claim 8 is rejected as allegedly indefinite for the use of the phrase "peptide fragment derived from". The rejection alleges that it is unclear how the claimed product has undergone any kind

of chemical modification as implied by the recitation "derived". The rejection alleges that one skilled in the art cannot ascribe a discrete and identifiable definition to the phase.

Without conceding the correctness of the rejection, and in an effort to expedite prosecution, the word "derived" in claim 8 has been replaced with "obtained". It is respectfully submitted that the change in wording overcomes the rejection.

The rejection of claim 9 is overcome by explicitly incorporating the method of claim 1 into claim 9.

Response to Section 102 Rejection

Claims 8-11 have been rejected as allegedly anticipated by US Patent No 6,048,530 to Srivastava. Reference is made to the comments set forth above under "Election / Restriction Requirement" in support of the novelty of the claimed special technical feature. The stress protein/peptide complexes defined in the Srivastava document are constitutively expressed rather than induced. As evidenced in Examples 4 and 5 of the specification, induced stress protein/peptide complexes are more immunogenic than constitutively expresses stress protein/peptide complexes, such as those described by Srivastava. Further the profile of peptides bound to an induced stress protein differs from those bound to constitutively expressed peptide (see Example 4 and Figures 1-3). This difference in immunogenicity by definition is manifestly due to a difference in structure. Thus, the constitutively expressed stress protein – peptide complexes of Srivastava are clearly not the same as the induced stress protein – peptide complexes of the present invention.

Reconsideration and withdrawal of the Section 102 rejection is respectfully submitted.

Conclusion

The claims of the application are believed in condition for allowance. An early action toward that end is earnest solicited.

Respectfully submitted

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